

**What is claimed is:**

1. A method of identifying an unknown bacterial bioagent comprising:

a) contacting nucleic acid from the bioagent with a pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences are between about 5 80-100% identical among different species of bioagents, wherein the sequences flank a variable nucleic acid sequence of the bioagent, and wherein the variable nucleic acid sequence exhibits no greater than about 5% identity among species and is between about 30 and 1000 nucleotides in length;

b) amplifying the variable nucleic acid sequence to produce a first amplification 10 product;

c) determining the molecular mass or base composition of the first amplification product;

d) comparing the molecular mass or base composition of the first amplification product to one or more calculated or measured molecular masses or base compositions of 15 amplification products of bacterial bioagents; and

e) performing steps a)-d) using at least one different oligonucleotide primer pair to obtain at least a second amplification product and comparing the results to one or more molecular masses or base compositions of amplification products of bacterial bioagents wherein at least one match determines the identity of the unknown bacterial bioagent.

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2. The method of claim 1 wherein the amplifying step comprises polymerase chain reaction.

3. The method of claim 1 wherein the amplifying step comprises ligase chain reaction 25 or strand displacement amplification.

4. The method of claim 1 wherein the nucleic acid encodes ribosomal RNA.

5. The method of claim 1 wherein the nucleic acid encodes RNase P or an RNA- 30 dependent RNA polymerase.

6. The method of claim 1 wherein the amplification product is ionized prior to molecular mass determination.

7. The method of claim 1 further comprising the step of isolating nucleic acid from the bioagent prior to contacting the nucleic acid with the pair of oligonucleotide primers.

8. The method of claim 1 wherein the one or more molecular masses or base compositions are contained in a database.

9. The method of claim 1 wherein the amplification product is ionized by electrospray ionization, matrix-assisted laser desorption, or fast atom bombardment.

10. The method of claim 1 wherein the molecular mass or base composition is determined by mass spectrometry.

11. The method of claim 10 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.

12. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

13. The method of claim 1 wherein the oligonucleotide primer comprises a base analog at positions 1 and 2 of each triplet within the primer, wherein the base analog binds with increased affinity to its complement compared to the native base.

14. The method of claim 13 wherein the primer comprises a universal base at position 3 of each triplet within the primer.

15. The method of claim 13 wherein the base analog is 2,6-diaminopurine, propyne T, propyne G, propyne C, phenoxazines, or G-clamp.

16. The method of claim 13 wherein the universal base is inosine, guanidine, uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, or 1-(2-deoxy- $\beta$ -D-ribofuranosyl)-imidazole-4-carboxamide.

17. The method of claim 1 wherein the sequences to which the pair of oligonucleotide primers hybridize are present within a gene involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, or secretion.

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18. The method of claim 1 wherein the sequences to which oligonucleotide primers hybridize are between about 90-100% identical among different species of bioagents.

19. The method of claim 1 wherein the sequences to which oligonucleotide primers hybridize are between about 95-100% identical among different species of bioagents.

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20. A method of identifying an unknown bacterial bioagent comprising:

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a) contacting nucleic acid from the bioagent with a pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences flank a variable nucleic acid sequence of the bioagent, and wherein the variable nucleic acid sequence exhibits no greater than about 5% identity among species and is between about 30 and 1000 nucleotides in length;

b) amplifying the variable nucleic acid sequence to produce a first amplification product;

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c) determining the molecular mass or base composition of the first amplification product;

d) comparing the molecular mass or base composition of the first amplification product to one or more calculated or measured molecular masses or base compositions of amplification products of bacterial bioagents; and

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e) performing steps a)-d) using at least one different oligonucleotide primer pair to obtain at least a second amplification product and comparing the results to one or more molecular masses or base compositions of amplification products of bacterial bioagents wherein at least one match determines the identity of the unknown bacterial bioagent..

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21. The method of claim 20 wherein the amplifying step comprises polymerase chain reaction.

22. The method of claim 20 wherein the amplifying step comprises ligase chain reaction or strand displacement amplification.
23. The method of claim 20 wherein the nucleic acid encodes ribosomal RNA.
- 5 24. The method of claim 20 wherein the nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
25. The method of claim 20 wherein the amplification product is ionized prior to  
10 molecular mass determination.
26. The method of claim 20 further comprising the step of isolating nucleic acid from the bioagent prior to contacting the nucleic acid with the pair of oligonucleotide primers.
- 15 27. The method of claim 20 wherein the one or more molecular masses or base compositions are contained in a database.
28. The method of claim 20 wherein the amplification product is ionized by electrospray ionization, matrix-assisted laser desorption, or fast atom bombardment.
- 20 29. The method of claim 20 wherein the molecular mass or base composition is determined by mass spectrometry.
30. The method of claim 29 wherein the mass spectrometry is Fourier transform ion  
25 cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.
31. The method of claim 20 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than  
30 adenosine, thymidine, guanosine or cytidine.

32. The method of claim 20 wherein the oligonucleotide primer comprises a base analog at positions 1 and 2 of each triplet within the primer, wherein the base analog binds with increased affinity to its complement compared to the native base.
- 5 33. The method of claim 32 wherein the primer comprises a universal base at position 3 of each triplet within the primer.
34. The method of claim 33 wherein the base analog is 2,6-diaminopurine, propyne T, propyne G, propyne C, phenoxazines, or G-clamp.
- 10 35. The method of claim 33 wherein the universal base is inosine, guanidine, uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, or 1-(2-deoxy- $\beta$ -D-ribofuranosyl)-imidazole-4-carboxamide.
- 15 36. The method of claim 20 wherein the sequences to which the pair of oligonucleotide primers hybridize are present within a gene involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, or secretion.
- 20 37. A method of identifying an unknown bacterial bioagent comprising:
- a) contacting nucleic acid from the bioagent with a pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences are between about 80-100% identical among different species of bioagents, and wherein the sequences flank a variable nucleic acid sequence of the bioagent which is between about 30 and 1000
  - 25 nucleotides in length;
  - b) amplifying the variable nucleic acid sequence to produce a first amplification product;
  - c) determining the molecular mass or base composition of the first amplification product;
  - 30 d) comparing the molecular mass or base composition of the first amplification product to one or more calculated or measured molecular masses or base compositions of amplification products of bacterial bioagents; and

e) performing steps a)-d) using at least one different oligonucleotide primer pair to obtain at least a second amplification product and comparing the results to one or more molecular masses or base compositions of amplification products of bacterial bioagents wherein at least one match determines the identity of the unknown bacterial bioagent

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38. The method of claim 37 wherein the amplifying step comprises polymerase chain reaction.

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39. The method of claim 37 wherein the amplifying step comprises ligase chain reaction or strand displacement amplification.

40. The method of claim 37 wherein the nucleic acid encodes ribosomal RNA.

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41. The method of claim 37 wherein the nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

42. The method of claim 37 wherein the amplification product is ionized prior to molecular mass determination.

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43. The method of claim 37 further comprising the step of isolating nucleic acid from the bioagent prior to contacting the nucleic acid with the pair of oligonucleotide primers.

44. The method of claim 37 wherein the one or more molecular masses or base compositions are contained in a database.

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45. The method of claim 37 wherein the amplification product is ionized by electrospray ionization, matrix-assisted laser desorption, or fast atom bombardment.

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46. The method of claim 37 wherein the molecular mass or base composition is determined by mass spectrometry.

47. The method of claim 46 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.

5 48. The method of claim 37 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

10 49. The method of claim 37 wherein the oligonucleotide primer comprises a base analog at positions 1 and 2 of each triplet within the primer, wherein the base analog binds with increased affinity to its complement compared to the native base.

50. The method of claim 49 wherein the primer comprises a universal base at position 3 of each triplet within the primer.

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51. The method of claim 49 wherein the base analog is 2,6-diaminopurine, propyne T, propyne G, propyne C, phenoxazines, or G-clamp.

20 52. The method of claim 50 wherein the universal base is inosine, guanidine, uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, or 1-(2-deoxy- $\beta$ -D-ribofuranosyl)-imidazole-4-carboxamide.

25 53. The method of claim 37 wherein the sequences to which the pair of oligonucleotide primers hybridize are present within a gene involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, or secretion.

54. The method of claim 37 wherein the sequences to which oligonucleotide primers hybridize are between about 90-100% identical among different species of bioagents.

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55. The method of claim 37 wherein the sequences to which oligonucleotide primers hybridize are between about 95-100% identical among different species of bioagents.

56. A method of identifying an unknown bacterial bioagent comprising:
- a) contacting nucleic acid from the bioagent with a pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences flank a variable nucleic acid sequence of the bioagent that is between about 30 and 1000 nucleotides in length;
  - b) amplifying the variable nucleic acid sequence to produce a first amplification product;
  - c) determining the molecular mass of the first amplification product;
  - d) comparing the molecular mass of the first amplification product to one or more calculated or measured molecular masses of amplification products of bacterial bioagents; and
  - e) performing steps a)-d) using at least one different oligonucleotide primer pair to obtain at least a second amplification product and comparing the results to one or more molecular masses of amplification products of bacterial bioagents wherein at least one match determines the identity of the unknown bacterial bioagent.
57. The method of claim 56 wherein the amplifying step comprises polymerase chain reaction.
58. The method of claim 56 wherein the amplifying step comprises ligase chain reaction or strand displacement amplification.
59. The method of claim 56 wherein the nucleic acid encodes ribosomal RNA.
60. The method of claim 56 wherein the nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.
61. The method of claim 56 wherein the amplification product is ionized prior to molecular mass determination.
62. The method of claim 56 further comprising the step of isolating nucleic acid from the bioagent prior to contacting the nucleic acid with the pair of oligonucleotide primers.



63. The method of claim 56 wherein the one or more molecular masses or base compositions are contained in a database.
64. The method of claim 56 wherein the amplification product is ionized by electrospray ionization, matrix-assisted laser desorption, or fast atom bombardment.
65. The method of claim 56 wherein the molecular mass or base composition is determined by mass spectrometry.
66. The method of claim 65 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.
67. The method of claim 56 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.
68. The method of claim 56 wherein the oligonucleotide primer comprises a base analog at positions 1 and 2 of each triplet within the primer, wherein the base analog binds with increased affinity to its complement compared to the native base.
69. The method of claim 68 wherein the primer comprises a universal base at position 3 of each triplet within the primer.
70. The method of claim 68 wherein the base analog is 2,6-diaminopurine, propyne T, propyne G, propyne C, phenoxazines, or G-clamp.
71. The method of claim 68 wherein the universal base is inosine, guanidine, uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, or 1-(2-deoxy- $\beta$ -D-ribofuranosyl)-imidazole-4-carboxamide.
72. The method of claim 56 wherein the sequences to which the pair of oligonucleotide primers hybridize are present within a gene involved in translation, replication,

recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, or secretion.

73. The method of claim 56 wherein the variable nucleic acid sequence of the bioagent  
5 is no more than about 50-250 nucleotides.

74. The method of claim 56 wherein the variable nucleic acid sequence of the bioagent  
is no more than about 60-100 nucleotides.